FEBS Letters 579 (2005) 3596–3600 FEBS 29666

Cooperative effect of hydrophobic and electrostatic forces on alcohol-induced α-helix formation of α_1-acid glycoprotein

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Received 11 April 2005; revised 14 May 2005; accepted 16 May 2005

Available online 8 June 2005

Edited by Maurice Montal

Abstract **α_1-Acid glycoprotein (AGP) is a serum glycoprotein that mainly binds basic drugs. Previous reports have shown that AGP converts from a β-sheet to an α-helix upon interaction with biomembranes. In the current studies, we found that alkanols, diols, and halogenols all induce this conformational change. Increased length and bulkiness of the hydrocarbon group and the presence of a halogen atom promoted this conversion, whereas the presence of a hydroxyl group inhibited it. Moreover, the effect was dependent on the hydrophobic and electrostatic properties of the alcohols. These results indicate that, in a membrane environment, hydrophobic and electrostatic factors cooperatively induce the transition of AGP from a β-sheet to an α-helix.**

Keywords: α_1-Acid glycoprotein; Alcohol; α-Helix formation; Conformational transition

1. Introduction

α_1-Acid glycoprotein (AGP), a member of the lipocalin family, is a polypeptide with two internal disulfide bonds and five carbohydrate chains that account for ~40% of its total mass (36 kDa) [1,2]. It is a major binding protein for neutral and basic ligands [3–5]. Although the three-dimensional structure and biological functions are still unknown, circular dichroism (CD) measurements [6] and molecular modeling [7] have revealed that this protein is mostly made up of β-sheets in aqueous solution.

It is widely accepted that membrane transport of a drug depends on the free drug concentration in solution. However, because this hypothesis does not fully explain the uptake mechanism for some AGP-binding drugs, a protein-mediated uptake system has been proposed [8–11]. In such a system, structural changes in the protein due to interaction with membrane surfaces decrease the drug-binding capacity. We previously reported that the interaction between AGP and model biomembranes (reverse micelles and liposomes) results in a unique conformational transition (β-sheet to α-helix) and a decrease in ligand-binding capacity [12,13]. Other studies of AGP interacting with vesicles [14] and liposomes [15] also support the conclusion that AGP interacts with the membrane in circulation.

A part of this interaction is due to the fact that the membrane potential decreases the local pH relative to the bulk solution [16]. The difference in pH has been experimentally determined to be 1.6 pH units, and the calculated value reaches 2.7 pH units [17]. Indeed, there are several reports that proteins, including other lipocalins, undergo structural and functional changes under mild acidic conditions on the membrane surface [18–21]. Also, Bychkova et al. [22] reported that cytochrome *c*, a mitochondrial protein, has a molten globule state in the presence of 40% methanol (MeOH) at pH 4.0, a condition that mimics the acidic conditions and low dielectric constant of the membrane surface environment.

There are other reports that a protein with high propensity for α-helix formation can easily convert to the α-helix form at pH 2.0 in the presence of various alcohols [23–26]. In addition, Kodicek et al. [27] reported that AGP heated in the presence of methanol (MeOH) forms a similar α-helix structure. These reports suggested that this effect was due to the hydrophobic force of alcohol and this force might induce the α-helix formation in AGP. On the other hand, the factors in such conformational transition of AGP are not elucidated.

In the present study, we used various alcohols (alkanols, diols, and halogenols) to investigate the mechanism of the α-helix formation in AGP and use of these alcohols allowed examination of how negative charge on the membrane surface and hydrophobic interaction inside the membrane affects the α-helix formation in AGP.

2. Materials and methods

2.1. Materials

AGP (Cohn Fraction VI) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All alcohols were purchased form Nacalai Tesque (Kyoto, Japan). All other chemicals and solvents were of analytical grade.

2.2. Measurement of CD spectra

CD spectra were recorded in a 1-mm path length cell with a JASCO J-720 spectropolarimeter using 10 μM AGP in 20 mM sodium acetate buffer, pH 4.0. The data were expressed as the molar residue ellipticity [θ] at 222 nm, which is an index of α-helix content.

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Abbreviations: The abbreviations used for the alcohols are summarized in Table 1; α_1-acid glycoprotein; CD, circular dichroism; ASA, solvent-accessible surface area; ASA (T), total ASA; rASA (H), relative ASA of hydrophobic region; rASA (N), relative ASA of negative charge region; rASA (P), relative ASA of positive charge region; HE_{50}, concentration of alcohol needed to form 50% α-helix structure

0014-5793/$30.00
doi:10.1016/j.febslet.2005.05.044

2.3. Calculation of m values

To analyze the effects of alcohols on AGP, we assumed a two-state transition between the β-sheet and the α-helix states. The equilibrium constant for the conformational transition, K, is defined as $K = [H]/[B]$, where [H] and [B] are the concentrations of the α-helix and β-sheet states, respectively. The free energy change (ΔG_f) for formation of the helical state is calculated according to following equation:

$$\Delta G_f = -RT \ln K,$$

where R is the gas constant, and T is the temperature in Kelvin. We assumed a linear dependence of ΔG_f upon the alcohol concentration ([alcohol]):

$$\Delta G_f = \Delta G_0 - m[\text{alcohol}],$$

where ΔG_0 is the ΔG_f value in the absence of alcohol, and m is a measure of the dependence of ΔG_f on the concentration of alcohol.

Least squares curve fitting was done using the MULTI program [28]. The solvent-accessible surface areas (total ASA [ASA (T)], hydrophobic ASA [ASA (H)], negative charge ASA [ASA (N)], and positive charge ASA [ASA (P)]) of alcohols were estimated using the Molecular Operating Environment (Chemical Computing Group Inc., Canada). ASA (H)/ASA (T), ASA (N)/ASA (T) and ASA (P)/ASA (T) were represented as relative ASA (H) [rASA (H)], relative ASA (N) [rASA (N)] and relative ASA (P) [rASA (P)], respectively (see Table 1).

3. Results

3.1. Effects of alkanols on α-helix formation in AGP

Based on a previous report that 40% methanol can induce α-helix formation at pH 4.0 [22], we investigated the effect of hydrocarbon groups in alcohols on the structure of AGP (Fig. 1A). The [θ] value at 222 nm, an index of α-helix content, showed saturation at lower concentrations as the alkyl chain length was increased. This demonstrated that the capacity to induce α-helix formation was increased as the hydrocarbon chain length was increased. However, there did not seem to be a difference in the effects of various alkanols on the α-helix content of AGP. Furthermore, 1-propanol, which has a straight chain, was more effective in promoting α-helix formation than 2-propanol, which has a branched chain.

3.2. Effects of diols on α-helix formation in AGP

Next, to determine the role of the hydroxyl group, we examined the effects of several diols on the α-helix content. As in the case of the alkanols, transition to an α-helix structure was observed at lower concentrations as the chain length was increased (Fig. 1B). However, the effect was weaker than in the case of the alkanols. This result suggests that the hydroxyl group of the alcohol only plays a role in their dissolution in water and that the induction of α-helix formation is due to the hydrocarbon moiety.

3.3. Effects of halogenols on α-helix formation in AGP

In addition, we examined the effect of halogen moieties by using several halogenols (Fig. 1C). The [θ] value at 222 nm in 2-chloroethanol (ClEtOH) showed the transition and saturation at lower concentrations than for the alkanols and diols, and caused a remarkable increase in the α-helix content. In contrast, the effect of 2-fluoroethanol (FEtOH) was weaker than that of ethanol. Bromoethanol could not be used because of phase separation. Alternatively, 2,2,2-trifluoroethanol (TFE) and 1,1,1,3,3,3-hexafluoro-2-ethanol (HFIP) was used. It was observed that these alcohols strongly promote an α-helix formation in AGP. These results imply that the presence of multiple F atoms increases the effectiveness markedly, although the F atom itself is not so potent and halogen atoms markedly increase the ability of the solvent to induce α-helix formation in AGP.

3.4. Effects of hydrophobic force and negative charge on α-helix formation in AGP

Based on the findings with various alcohols, it appeared likely that the hydrophobicity and negative charge of the alcohol participate in the induction of α-helix formation in AGP. Thus, we estimated rASA (H), rASA (P), and rASA (N), respectively, which do not depend on the molecular weight or ASA (T), to evaluate the properties of the alcohols. The effects of the alcohols on AGP were evaluated using with the m value and the α-helix effect$_{50}$ (HE_{50}), which is the concentration (%) resulting in a 50% transition from a β-sheet to an α-helix. For all kinds of alcohols, HE_{50} appears to more accurately describe the effects of the alcohol than the m value because the m value depends on maximum α-helix content. In Fig. 2, correlations between HE_{50} and rASA were presented. In alkanols, a good correlation was observed between the HE_{50} and rASA (H) ($r = 0.8849$), suggesting that hydrophobic interactions are involved in α-helix formation in AGP at pH

Table 1
The m, HE_{50}, rASA (H), rASA (N) and rASA (P) values for various alcohols

	Abbreviation used	m (J mol^{-1} M^{-1})	HE_{50}	rASA (H)	rASA (N)	rASA (P)
Alkanols						
Methanol	MeOH	573.7	35.1	0.41	0.17	0.42
Ethanol	EtOH	751.1	28.8	0.67	0.11	0.21
1-Propanol	1-PrOH	952.2	21.8	0.72	0.10	0.17
2-Propanol	2-PrOH	789.8	25.7	0.78	0.09	0.12
Diols						
1,2-Ethanediol	1,2-Et(OH)$_2$	328.9	67.4	0.42	0.21	0.38
1,2-Propanediol	1,2-Pr(OH)$_2$	381.2	54.5	0.58	0.17	0.25
1.4-Butanediol	1,4-Bu(OH)$_2$	393.2	49.3	0.64	0.12	0.24
2,3-Butanediol	2,3-Bu(OH)$_2$	469.5	51.3	0.68	0.13	0.18
Halogenols						
2-Fluoroethanol	FEtOH	625.7	37.1	0.43	0.28	0.28
2-Chloroethanol	ClEtOH	397.4	26.6	0.38	0.42	0.20
2,2,2-Trifluoroethanol	TFE	545.3	23.1	0.20	0.60	0.20
1,1,1,3,3,3-Hexafluoro-2-propanol	HFIP	1004.9	5.8	0.07	0.79	0.12

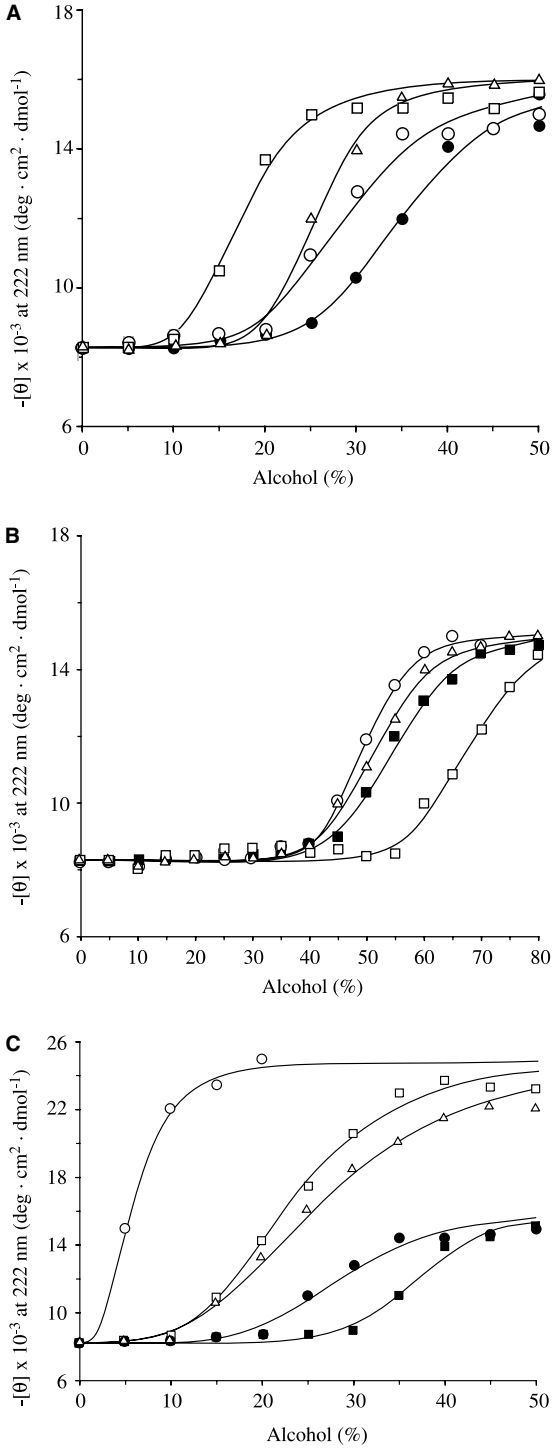

Fig. 1. Effects of alkanols (A), diols (B), and halogenols (C) on α-helix formation in AGP. The α-helix content of AGP was monitored at pH 4.0 by measuring the ellipticity at 222 nm. Abbreviations are as follows: in panel A, MeOH (●), EtOH (○), 1-PrOH (□), and 2-PrOH (△); in panel B, $Et(OH)_2$ (□), 1,2-$Pr(OH)_2$ (■), 2,3-$Bu(OH)_2$ (△), and 1,4-$Bu(OH)_2$ (○); and in panel C, EtOH (●), FEtOH (■), ClEtOH (△), TFE (□), and HFIP (○). The lines were drawn using the MULTI program [28].

4.0 (Fig. 2A). For diols, rASA (H) showed significant correlations with the HE_{50} ($r = 0.9685$, $P < 0.05$). This result also indicates that hydrocarbon group contributes to α-helix formation in AGP, although the effect itself was weaker than that of alkanols (Fig. 2A). On the other hand, for halogenols we found a significant correlation between HE_{50} and rASA (N) ($r = 0.9702$, $P < 0.05$), which explains the strong effect of the negative charge on α-helix formation in AGP (Fig. 2B).

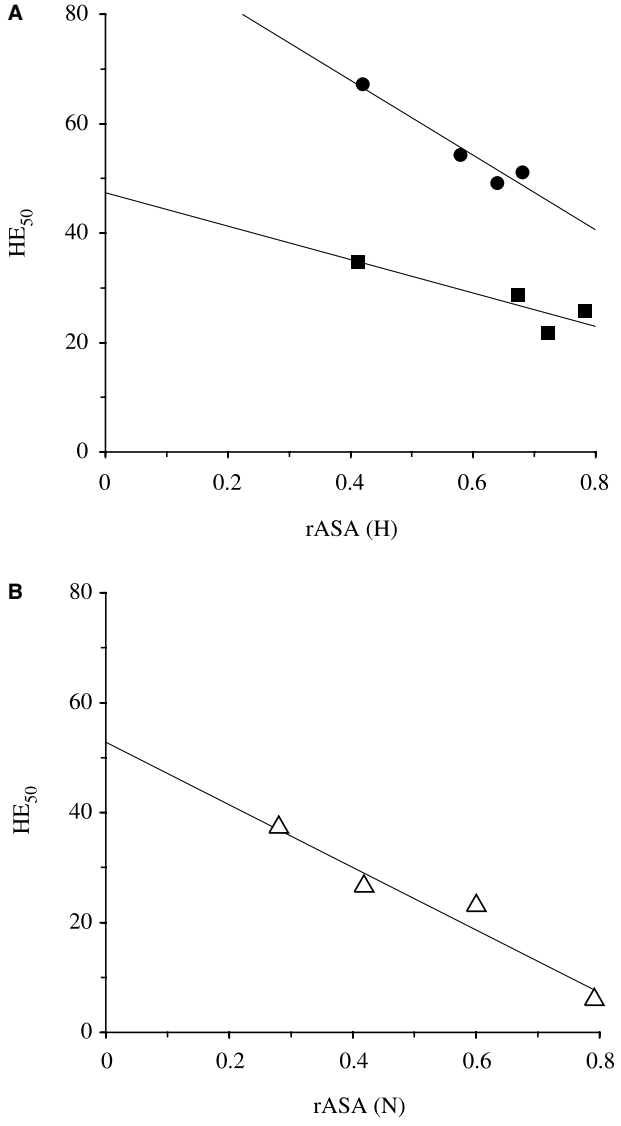

Fig. 2. Correlations between the values of HE_{50} and rASA. A and B show the correlations between HE_{50} and rASA (H) or rASA (N), respectively. Each symbol represented as follow: alkanol (●), diol (■) and halogenol (△).

3.5. Effect of halogen (Cl^-) on α-helix formation in AGP

The significant correlation between HE_{50} and rASA (N) in halogenols indicates that halogen is an important factor in the induction of α-helix formation in AGP. Previously, we reported that NaCl (1 M) induced an α-helix structure at pH 2.0 [12]. To examine the effect of the halogen, Cl^-, on the α-helix formation, CD spectra was monitored in the presence of HCl and NaCl (0–1 M) at pH 2.0 (Fig. 3). Interestingly, addition of HCl induced the α-helix structure, although AGP was in an unfolded state at pH 2.0. Moreover, NaCl also showed a significant ability to induce α-helix formation. These results suggested that halogen is a key factor in the induction of α-helix

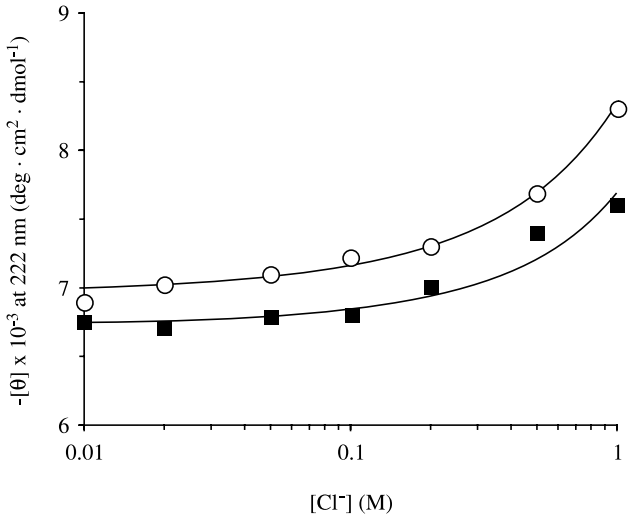

Fig. 3. Effect of halogen (Cl^-) on α-helix formation in AGP. The α-helix content of AGP was monitored at pH 2.0 by measuring the ellipticity at 222 nm. Abbreviations are as follows: ○, NaCl; and ■, HCl. The lines were drawn using the MULTI program [28].

formation by halogenols and that negative charge acts cooperatively with hydrophobicity to induce α-helix structure formation.

4. Discussion

Previously, we reported that AGP interacts with biomembrane models, including liposomes and reverse micelles, and that this interaction is followed by conformational transition from a β-sheet form to an α-helix form [12,13]. This conformational transition may be linked not only to ligand-binding capacity but also to intracellular biological activity [29–33], but which factors participate in such a conformational transition has not been determined. Generally, it has been thought that following factors are related to the interaction between proteins and lipid membranes: (1) negative charge of the membrane surface; (2) a mild acidic environment on the membrane surface and (3) a hydrophobic membrane interior [17]. However, it is difficult to evaluate the role of these factors in the conformational transition using liposomes and reverse micelles separately.

In the present study, to clarify the mechanism by which AGP undergoes a transition from an α-helix to a β-sheet structure, we examined the effects of various alcohols. Both Alkanol and diol showed larger values of rASA (H) than rASA (N) and rASA (P) and the abilities of alkanols and diols to induce formation of an α-helix structure were dependent on the length of their hydrocarbon groups. These HE_{50} values correlated well with the rASA (H). These results indicate that hydrophobic force is a key factor for inducing and stabilizing the α-helix structure. The effects of diols were weaker than other alcohols because the hydroxyl group weakened the hydrophobicity. On the other hand, the HE_{50} values of halogenols correlated significantly with the rASA (N), implying that the α-helix formation in the presence of alcohols is due to the cooperative action of hydrophobic and electrostatic forces. The strong effect of halogenols may be due to their high rASA (N). This hypothesis is also supported by the finding that HCl and NaCl induced α-helix formation in AGP even at pH 2.0. Under acidic conditions, it appears that hydrophobic interaction between the hydrocarbon group of the alcohol and the exposed hydrophobic region of AGP induces formation of the α-helix structure. Kodicek et al. [27] reported that AGP formed a similar α-helix structure in the presence of MeOH at high temperature. We also found that the conformational structure of AGP perturbed at high temperature (data not shown). In addition, suppression of the electrostatic repulsion of positive charges in AGP by the negative charge of the halogen might enhance the hydrophobic interaction. Although HCl and NaCl also induced α-helix formation, the effect was weaker than that of alcohols. These results indicate that there is a slight increase in the extent of α-helix conformation when AGP interacts with the membrane surface, and then the peptide inserts into the membrane interior, where it forms an α-helix-rich structure.

In the present study, we found that hydrophobic and electrostatic forces cooperatively promote α-helix formation in AGP. The biological function of AGP is not clear, but some reports suggest that it has intracellular activities [29–33]. Given that AGP is a plasma protein, the conformational transition to an α-helix structure may be critical for its activity.

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